Electronic Supplementary Information

A rapid genomic DNA extraction method and its combination with helicase dependent amplification for the detection of genetically modified maize

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1. Materials and Methods

1.1 Extraction procedures

CTAB extraction protocol

The extraction was carried out based on the maize DNA isolation method proposed by the Community Reference Laboratory for GM Food and Feed, 2007. Briefly, genomic DNA was extracted by performing a thermal lysis (65ºC, 1h) of the sample with the extraction buffer (1.4 M NaCl, 2% (w/v) cetyltrimethylammonium bromide (CTAB), 0.1M Tris-Base (pH=8), 0.02M EDTA (pH=8) and 1% polyvinyl pyrrolidone 40000). DNA was later precipitated with the precipitation buffer (1% (w/v) CTAB, 0.05 M Tris (pH=8), 0.01 M EDTA (pH=8)) and washed with 70% ethanol. Resuspension of the DNA was done afterwards in 10 mM Tris buffer at 65ºC for 20 min and stored at -20ºC until further use. All reagents were from Roth (Karlsruhe, Germany). Each extraction was done in triplicates.

Wizard® Genomic DNA Purification Kit

The protocol was carried out according to the manufacturer. Briefly, 40mg of ground maize powder were mixed with the nuclei lysis solution and incubated at 65ºC for 15 min. RNase solution was afterwards added and further incubated at 37ºC for 15 min. The protein precipitation solution was then added and the mixture was centrifuged at 15000 g. The supernatant was later removed and mixed with room temperature isopropanol. After centrifugation, the pellet formed was washed with 70% ethanol and air dried. The genomic DNA extracted was rehydrated with DNA Rehydration Solution and incubated at 65ºC for 1 h. Solutions were then stored at -20ºC until further use. Each extraction was done in triplicates.

2. Results

2.1 Optimization of incubations times with proteinase K

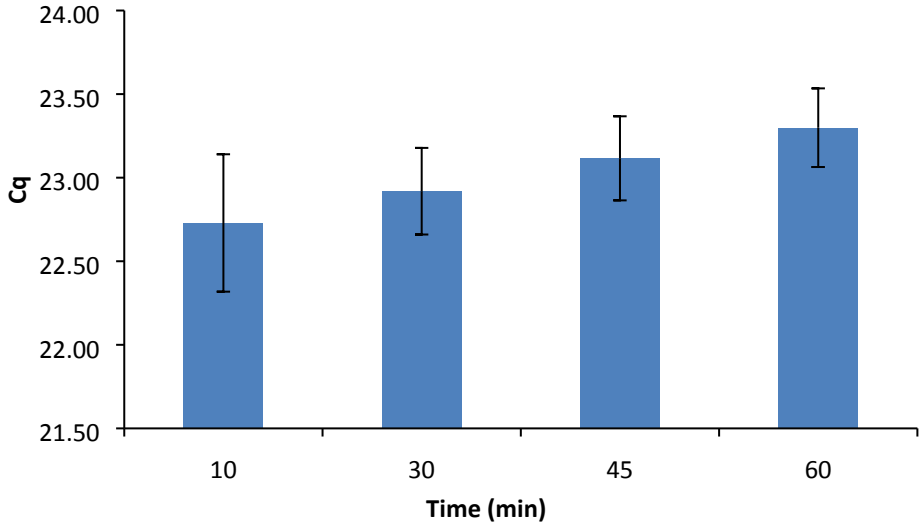

Figure 1. Optimization of incubation times when digesting the samples with proteinase K.

2.2 Optimization of the best temperature of extraction

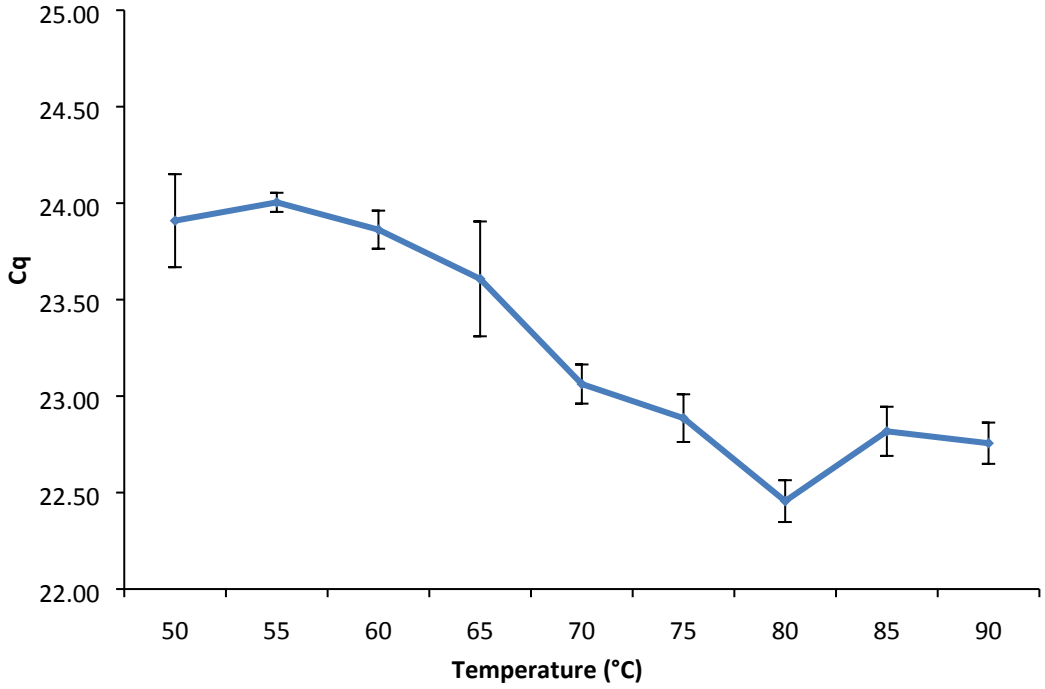

Figure 2. Screening for the best temperature of extraction.

2.3 Addition of chemicals to the sodium phosphate buffer

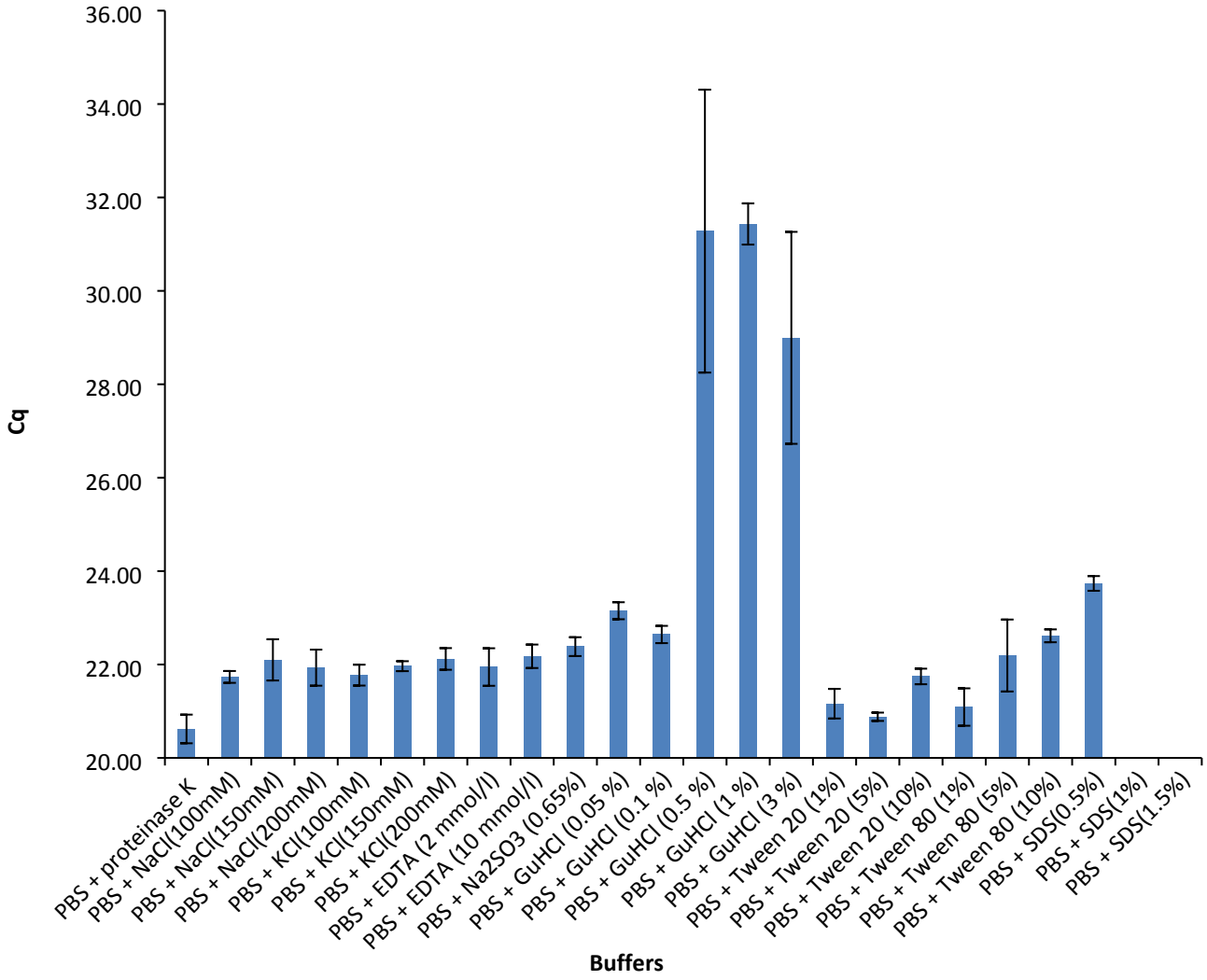

Figure 3. Influence of the usage of different chemicals in the sodium phosphate buffer in the Cq value of the extracts.

2.4 Pretreatment of samples with solvents

Table 1. Results from a pretreatment of the maize powder samples with solvents.

Sample	Cq ± STD
CH_3Cl	23.87 ± 0.68
CH_3Cl-EtoAc (1:1)	24.22 ± 0.86
EtoAc	24.82 ± 0.99